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10/673,786	09/30/2003	Valery Zavenovich Akhverdian	US-115	7880

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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/673,786
Filing Date: September 30, 2003
Appellant(s): AKHVERDIAN ET AL.

Shelly Guest Cermak
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 9/5/2007 appealing from the Office action mailed 5/11/2007.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

The amendment after final rejection filed on 4/18/2007 has been entered.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

5,175,107	DEBABOV et al.	12-1992
6,319,696	KISHINO et al.	11-2001
WO 87/00202	EDWARDS et al.	1-1987

EP 0219027	KATSUMATA et al.	4-1987
6,040,160	KOJIMA et al.	3-2000

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 12, 15-16, 19, 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Katsumata et al. (EP 0219027 published 4/22/1987; cited in the IDS) in view of Debabov et al. (U.S. Patent No. 5175107 issued on 12/29/1992; cited in the IDS), Edwards et al. (WO 87/00202 published on 1/15/1987; cited in the IDS), and further in view of Kishino et al. (U.S. Patent No. 6319696 issued on 11/20/2001).

Katsumata et al. teach a process for the production of L-threonine by a *C. glutamicum* cell wherein said *C. glutamicum* cell is transformed with a plasmid comprising a *C. glutamicum* gene

encoding aspartate aminotransferase (Abstract; page 17, line 26-page 19, line 31; page 20, line 25-page 21, line 20). The *C. glutamicum* aspartate aminotransferase of Katsumata et al. has been identified as corresponding to EC 2.6.1.1 (official name aspartate aminotransferase) and has been labeled by Katsumata et al. as AAT throughout the document (page 1, lines 21-27). Katsumata et al. do not teach production of L-threonine by an *Escherichia* bacterium or the aspartate aminotransferase of SEQ ID NO: 2.

Debabov et al. teach an *E. coli* cell which is a high threonine producer (B-3996; Abstract; Figure 1; column 1, lines 20-41, column 1, line 68-column 2, line 7; claim 1) which has been deposited on November 19, 1987 in the collection of microorganism cultures at the USSR Antibiotic Research Institute Moscow (column 1, lines 56-63). This is the same *E. coli* strain B-3996 disclosed in the specification of the instant application (pages 11-12, paragraph [0050]) as having enhanced expression of a mutant aspartokinase homoserine dehydrogenase 1 resistant to feedback inhibition by threonine (thrA), a gene encoding a homoserine kinase (thrB), a gene encoding a threonine synthase (thrC), and a gene encoding a transmembrane protein (rhtA). This is also the same *E. coli* strain disclosed in the specification under Examples 1-2, where plasmids containing the *E. coli* aspC gene were introduced. See, for example, Table 1 of the specification, first column, strain. Debabov et al. do not teach the aspartate aminotransferase of SEQ ID NO: 2.

Edwards et al. teach the production of aromatic amino acids (Abstract) by overexpressing several genes including the *E. coli* aspC gene (Example 8, page 36, line 25-page 37, line 31). Edwards et al. discloses cloning the *E. coli* aspC gene (page 18, lines 21-34) whose sequence is shown in Chart 6 (page 21). The *E. coli* aspC disclosed by Edwards et al. comprises SEQ ID NO: 1 and encodes the polypeptide of SEQ ID NO: 2. Edwards et al. do not teach production of L-threonine or an *E. coli* cell which is further modified to increase the expression of a mutant gene encoding an aspartokinase homoserine dehydrogenase 1 resistant to feedback inhibition by threonine, a gene encoding a homoserine kinase, a

gene encoding a threonine synthase, and/or a gene encoding a transmembrane protein. For the reader's convenience, the alignments are shown below:

```
RESULT 1
I08485
LOCUS       I08485                  1293 bp    DNA     linear   PAT 02-DEC-
1994
DEFINITION  Sequence 12 from Patent WO 8700202.
ACCESSION   I08485
VERSION     I08485.1  GI:588805
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (bases 1 to 1293)
  AUTHORS   Edwards,M.R., Taylor,P.P., Hunter,M.G. and Fotheringham,I.G.
  TITLE     COMPOSITE PLASMIDS FOR AMINO ACID SYNTHESIS
  JOURNAL   Patent: WO 8700202-A 12 15-JAN-1987;
FEATURES             Location/Qualifiers
     source          1. .1293
                     /organism="unknown"
                     /mol_type="unassigned DNA"
ORIGIN

  Query Match             100.0%;  Score 1191;  DB 6;  Length 1293;
  Best Local Similarity   100.0%;  Pred. No. 0;
  Matches 1191;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps
0;

Qy          1 ATGTTTGAGAACATTACCGCCGCTCCTGCCGACCCGATTCTGGGCCTGGCCGATCTGTTT 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         12 ATGTTTGAGAACATTACCGCCGCTCCTGCCGACCCGATTCTGGGCCTGGCCGATCTGTTT 71

Qy         61 CGTGCCGATGAACGTCCCGGCAAAATTAACCTCGGGATTGGTGTCTATAAAGATGAGACG 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         72 CGTGCCGATGAACGTCCCGGCAAAATTAACCTCGGGATTGGTGTCTATAAAGATGAGACG 131

Qy        121 GGCAAAACCCCGGTACTGACCAGCGTGAAAAAGGCTGAACAGTATCTGCTCGAAAATGAA 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        132 GGCAAAACCCCGGTACTGACCAGCGTGAAAAAGGCTGAACAGTATCTGCTCGAAAATGAA 191

Qy        181 ACCACCAAAAATTACCTCGGCATTGACGGCATCCCTGAATTTGGTCGCTGCACTCAGGAA 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        192 ACCACCAAAAATTACCTCGGCATTGACGGCATCCCTGAATTTGGTCGCTGCACTCAGGAA 251

Qy        241 CTGCTGTTTGGTAAAGGTAGCGCCCTGATCAATGACAAACGTGCTCGCACGGCACAGACT 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        252 CTGCTGTTTGGTAAAGGTAGCGCCCTGATCAATGACAAACGTGCTCGCACGGCACAGACT 311

Qy        301 CCGGGGGGCACTGGCGCACTACGCGTGGCTGCCGATTTCCTGGCAAAAAATACCAGCGTT 360
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Db        312 CCGGGGGGCACTGGCGCACTACGCGTGGCTGCCGATTTCCTGGCAAAAAATACCAGCGTT 371

Qy        361 AAGCGTGTGTGGGTGAGCAACCCAAGCTGGCCGAACCATAAGAGCGTCTTTAACTCTGCA 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        372 AAGCGTGTGTGGGTGAGCAACCCAAGCTGGCCGAACCATAAGAGCGTCTTTAACTCTGCA 431

Qy        421 GGTCTGGAAGTTCGTGAATACGCTTATTATGATGCGGAAAATCACACTCTTGACTTCGAT 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        432 GGTCTGGAAGTTCGTGAATACGCTTATTATGATGCGGAAAATCACACTCTTGACTTCGAT 491

Qy        481 GCACTGATTAACAGCCTGAATGAAGCTCAGGCTGGCGACGTAGTGCTGTTCCATGGCTGC 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        492 GCACTGATTAACAGCCTGAATGAAGCTCAGGCTGGCGACGTAGTGCTGTTCCATGGCTGC 551

Qy        541 TGCCATAACCCAACCGGTATCGACCCTACGCTGGAACAATGGCAAACACTGGCACAACTC 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        552 TGCCATAACCCAACCGGTATCGACCCTACGCTGGAACAATGGCAAACACTGGCACAACTC 611

Qy        601 TCCGTTGAGAAAGGCTGGTTACCGCTGTTTGACTTCGCTTACCAGGGTTTTGCCCGTGGT 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        612 TCCGTTGAGAAAGGCTGGTTACCGCTGTTTGACTTCGCTTACCAGGGTTTTGCCCGTGGT 671

Qy        661 CTGGAAGAAGATGCTGAAGGACTGCGCGCTTTCGCGGCTATGCATAAAGAGCTGATTGTT 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        672 CTGGAAGAAGATGCTGAAGGACTGCGCGCTTTCGCGGCTATGCATAAAGAGCTGATTGTT 731

Qy        721 GCCAGTTCCTACTCTAAAAACTTTGGCCTGTACAACGAGCGTGTTGGCGCTTGTACTCTG 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        732 GCCAGTTCCTACTCTAAAAACTTTGGCCTGTACAACGAGCGTGTTGGCGCTTGTACTCTG 791

Qy        781 GTTGCTGCCGACAGTGAAACCGTTGATCGCGCATTCAGCCAAATGAAAGCGGCGATTCGC 840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        792 GTTGCTGCCGACAGTGAAACCGTTGATCGCGCATTCAGCCAAATGAAAGCGGCGATTCGC 851

Qy        841 GCTAACTACTCTAACCCACCAGCACACGGCGCTTCTGTTGTTGCCACCATCCTGAGCAAC 900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        852 GCTAACTACTCTAACCCACCAGCACACGGCGCTTCTGTTGTTGCCACCATCCTGAGCAAC 911

Qy        901 GATGCGTTACGTGCGATTTGGGAACAAGAGCTGACTGATATGCGCCAGCGTATTCAGCGT 960
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        912 GATGCGTTACGTGCGATTTGGGAACAAGAGCTGACTGATATGCGCCAGCGTATTCAGCGT 971

Qy        961 ATGCGTCAGTTGTTCGTCAATACGCTGCAGGAAAAAGGCGCAAACCGCGACTTCAGCTTT1020
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        972 ATGCGTCAGTTGTTCGTCAATACGCTGCAGGAAAAAGGCGCAAACCGCGACTTCAGCTTT1031

Qy       1021 ATCATCAAACAGAACGGCATGTTCTCCTTCAGTGGCCTGACAAAAGAACAAGTGCTGCGT1080
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1032 ATCATCAAACAGAACGGCATGTTCTCCTTCAGTGGCCTGACAAAAGAACAAGTGCTGCGT1091

Qy       1081 CTGCGCGAAGAGTTTGGCGTATATGCGGTTGCTTCTGGTCGCGTAAATGTGGCCGGGATG1140
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1092 CTGCGCGAAGAGTTTGGCGTATATGCGGTTGCTTCTGGTCGCGTAAATGTGGCCGGGATG1151
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Qy       1141 ACACCAGATAACATGGCTCCGCTGTGCGAAGCGATTGTGGCAGTGCTGTAA 1191
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1152 ACACCAGATAACATGGCTCCGCTGTGCGAAGCGATTGTGGCAGTGCTGTAA 1202
```

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RESULT 1
I08485
LOCUS       I08485                  1293 bp    DNA     linear   PAT 02-DEC-
1994
DEFINITION  Sequence 12 from Patent WO 8700202.
ACCESSION   I08485
VERSION     I08485.1  GI:588805
KEYWORDS    .
SOURCE      Unknown.
  ORGANISM  Unknown.
            Unclassified.
REFERENCE   1  (bases 1 to 1293)
  AUTHORS   Edwards,M.R., Taylor,P.P., Hunter,M.G. and Fotheringham,I.G.
  TITLE     COMPOSITE PLASMIDS FOR AMINO ACID SYNTHESIS
  JOURNAL   Patent: WO 8700202-A 12 15-JAN-1987;
FEATURES             Location/Qualifiers
     source          1. .1293
                     /organism="unknown"
                     /mol_type="unassigned DNA"
ORIGIN
```

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Alignment Scores:
Pred. No.:              7.8e-149        Length:         1293
Score:                  2045.00         Matches:        396
Percent Similarity:     100.0%          Conservative:   0
Best Local Similarity:  100.0%          Mismatches:     0
Query Match:            100.0%          Indels:         0
DB:                     6               Gaps:           0

US-10-673-786A-2 (1-396) x I08485 (1-1293)

Qy          1 MetPheGluAsnIleThrAlaAlaProAlaAspProIleLeuGlyLeuAlaAspLeuPhe 20
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         12 ATGTTTGAGAACATTACCGCCGCTCCTGCCGACCCGATTCTGGGCCTGGCCGATCTGTTT 71

Qy         21 ArgAlaAspGluArgProGlyLysIleAsnLeuGlyIleGlyValTyrLysAspGluThr 40
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         72 CGTGCCGATGAACGTCCCGGCAAAATTAACCTCGGGATTGGTGTCTATAAAGATGAGACG 131

Qy         41 GlyLysThrProValLeuThrSerValLysLysAlaGluGlnTyrLeuLeuGluAsnGlu 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        132 GGCAAAACCCCGGTACTGACCAGCGTGAAAAAGGCTGAACAGTATCTGCTCGAAAATGAA 191

Qy         61 ThrThrLysAsnTyrLeuGlyIleAspGlyIleProGluPheGlyArgCysThrGlnGlu 80
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        192 ACCACCAAAAATTACCTCGGCATTGACGGCATCCCTGAATTTGGTCGCTGCACTCAGGAA 251
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Qy        81 LeuLeuPheGlyLysGlySerAlaLeuIleAsnAspLysArgAlaArgThrAlaGlnThr 100
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       252 CTGCTGTTTGGTAAAGGTAGCGCCCTGATCAATGACAAACGTGCTCGCACGGCACAGACT 311

Qy       101 ProGlyGlyThrGlyAlaLeuArgValAlaAlaAspPheLeuAlaLysAsnThrSerVal 120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       312 CCGGGGGGCACTGGCGCACTACGCGTGGCTGCCGATTTCCTGGCAAAAAATACCAGCGTT 371

Qy       121 LysArgValTrpValSerAsnProSerTrpProAsnHisLysSerValPheAsnSerAla 140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       372 AAGCGTGTGTGGGTGAGCAACCCAAGCTGGCCGAACCATAAGAGCGTCTTTAACTCTGCA 431

Qy       141 GlyLeuGluValArgGluTyrAlaTyrTyrAspAlaGluAsnHisThrLeuAspPheAsp 160
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       432 GGTCTGGAAGTTCGTGAATACGCTTATTATGATGCGGAAAATCACACTCTTGACTTCGAT 491

Qy       161 AlaLeuIleAsnSerLeuAsnGluAlaGlnAlaGlyAspValValLeuPheHisGlyCys 180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       492 GCACTGATTAACAGCCTGAATGAAGCTCAGGCTGGCGACGTAGTGCTGTTCCATGGCTGC 551

Qy       181 CysHisAsnProThrGlyIleAspProThrLeuGluGlnTrpGlnThrLeuAlaGlnLeu 200
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       552 TGCCATAACCCAACCGGTATCGACCCTACGCTGGAACAATGGCAAACACTGGCACAACTC 611

Qy       201 SerValGluLysGlyTrpLeuProLeuPheAspPheAlaTyrGlnGlyPheAlaArgGly 220
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       612 TCCGTTGAGAAAGGCTGGTTACCGCTGTTTGACTTCGCTTACCAGGGTTTTGCCCGTGGT 671

Qy       221 LeuGluGluAspAlaGluGlyLeuArgAlaPheAlaAlaMetHisLysGluLeuIleVal 240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       672 CTGGAAGAAGATGCTGAAGGACTGCGCGCTTTCGCGGCTATGCATAAAGAGCTGATTGTT 731

Qy       241 AlaSerSerTyrSerLysAsnPheGlyLeuTyrAsnGluArgValGlyAlaCysThrLeu 260
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       732 GCCAGTTCCTACTCTAAAAACTTTGGCCTGTACAACGAGCGTGTTGGCGCTTGTACTCTG 791

Qy       261 ValAlaAlaAspSerGluThrValAspArgAlaPheSerGlnMetLysAlaAlaIleArg 280
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       792 GTTGCTGCCGACAGTGAAACCGTTGATCGCGCATTCAGCCAAATGAAAGCGGCGATTCGC 851

Qy       281 AlaAsnTyrSerAsnProProAlaHisGlyAlaSerValValAlaThrIleLeuSerAsn 300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       852 GCTAACTACTCTAACCCACCAGCACACGGCGCTTCTGTTGTTGCCACCATCCTGAGCAAC 911

Qy       301 AspAlaLeuArgAlaIleTrpGluGlnGluLeuThrAspMetArgGlnArgIleGlnArg 320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       912 GATGCGTTACGTGCGATTTGGGAACAAGAGCTGACTGATATGCGCCAGCGTATTCAGCGT 971

Qy       321 MetArgGlnLeuPheValAsnThrLeuGlnGluLysGlyAlaAsnArgAspPheSerPhe 340
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       972 ATGCGTCAGTTGTTCGTCAATACGCTGCAGGAAAAAGGCGCAAACCGCGACTTCAGCTTT1031

Qy       341 IleIleLysGlnAsnGlyMetPheSerPheSerGlyLeuThrLysGluGlnValLeuArg 360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Db       1032 ATCATCAAACAGAACGGCATGTTCTCCTTCAGTGGCCTGACAAAAGAACAAGTGCTGCGT1091

Qy        361 LeuArgGluGluPheGlyValTyrAlaValAlaSerGlyArgValAsnValAlaGlyMet 380
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1092 CTGCGCGAAGAGTTTGGCGTATATGCGGTTGCTTCTGGTCGCGTAAATGTGGCCGGGATG1151

Qy        381 ThrProAspAsnMetAlaProLeuCysGluAlaIleValAlaValLeu 396
              ||||||||||||||||||||||||||||||||||||||||||||||||
Db       1152 ACACCAGATAACATGGCTCCGCTGTGCGAAGCGATTGTGGCAGTGCTG 1199
```

Kishino et al. teach production of L-threonine by culturing *E. coli* cells transformed with vectors that express *E. coli* genes encoding enzymes directly associated with the biosynthesis of L-threonine. Kishino et al. teach the use of low copy number vectors such as pBR322 (column 5, lines 36-40) and also teach that the *E. coli* strain B-3996 (described above) is a preferred strain for the production of L-threonine due to its high threonine yields (column 4, lines 7-9). Kishino et al. further teach the use of promoters to increase expression of a gene, such as lac, trp, and P_L (column 5, lines 42-44). Kishino et al. do not teach production of L-threonine by culturing an *E. coli* cell transformed with a vector comprising the nucleic acid of SEQ ID NO: 1.

Claims 12 and 19 are directed in part to a method for producing L-threonine wherein said method requires cultivating an *E. coli* cell modified to increase the expression of (1) a gene comprising nucleotides 1-1191 of SEQ ID NO: 1, (2) the *E. coli* thrA gene encoding an aspartokinase homoserine dehydrogenase I which is resistant to feedback inhibition, (3) the *E. coli* thrB gene, (4) the *E. coli* thrC gene, and (5) the *E. coli* rhtA gene, wherein the expression of the genes of (1)-(5) is increased by increasing the copy number of the genes or by placing said genes under the control of a potent promoter. Claims 15-16 are directed in part to the method of claim 12 wherein the expression of said genes is increased by increasing the copy number of said genes using a low copy number vector. Claim 23 is directed in part to the method of claim 12 wherein the potent promoter is selected from the group consisting of the lac promoter, trp promoter, trc promoter, P_R promoter and P_L promoter. The gene of SEQ ID NO: 1 contains 1191 nucleotides and encodes the polypeptide of SEQ ID NO: 2.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to transform the *E. coli* strain of Debabov et al. with the low copy number vector of Kishino et al., wherein said vector comprises the nucleic acid of Edwards et al. for the production of L-threonine. Also, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the *E. coli* strain of Debabov et al. in order to increase the expression of the nucleic acid of Edwards et al. by placing said nucleic acid under the control of the lac, trp, or P_L promoters, as taught by Kishino et al., for the production of L-threonine. A person of ordinary skill in the art is motivated to create the *E. coli* cell of Debabov, Kishino and Edwards and culture said cell to produce L-threonine in view of the fact that (1) Katsumata et al. teach the production of L-threonine by culturing a microorganism transformed with a vector comprising the endogenous gene encoding aspartate aminotransferase so that the level of aspartate aminotransferase is increased in that microorganism, (2) Kishino et al. teach that high threonine producers, such as the strain of Debabov et al., are preferred strains for L-threonine production, (3) the gene taught by Edwards et al. is the *E. coli* aspartate aminotransferase gene and is the preferred aspartate aminotransferase gene to express by the *E. coli* strain of Debabov et al. because it would not be recognized by the cell as foreign, (4) a low copy number vector or an inducible promoter such as lac, trp, or P_L would allow for better control of how much of the aspartate aminotransferase is produced and avoid intracellular instability, and (5) aspartate aminotransferase catalyzes the conversion of oxaloacetate to aspartate, which is a precursor of L-threonine.

One of ordinary skill in the art has a reasonable expectation of success at inserting the DNA of Edwards et al. in a low copy number vector, or placing such DNA under the control of the lac, trp or P_L promoters taught by Kishino et al., transform the *E. coli* strain of Debabov et al., and culture the resulting recombinant microorganism for the production of L-threonine since (1) cloning genes in low copy number vectors and placing genes under the control of inducible promoters such as lac, trp or P_L is well

known and widely practiced in the art, (2) transformation of *E. coli* cells is well known and widely used in the art, (3) increasing the levels of aspartate aminotransferase should result in an increase in aspartate, which in turn should result in increasing levels of L-threonine, and (4) Katsumata et al. teach L-threonine production by culturing a microorganism wherein the endogenous aspartate aminotransferase levels are increased. Therefore, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made.

(10) Response to Argument

On page 7 of the Brief, Appellant argues that many aminotransferases are known in the art and that their substrate specificities are different . Appellant refers to Exhibit A in support of the argument that there are 16 types of aminotransferases in *E. coli* and 14 types in *C. glutamicum*. Appellant submits that it is not clear from the prior art which aminotransferases are effective for increasing the production of L-threonine and asserts that it is unclear which type of aminotransferase is encoded by the *C. glutamicum* used by Katsumata et al.

The Examiner acknowledges that there are many types of aminotransferases known in the prior art. However, the Examiner disagrees with Appellant's contention that it is not clear from the teachings of Katsumata et al. as to which type of aminotransferase was encoded by the *C. glutamicum* gene used by Katsumata et al. The Examiner indicated in the Final action mailed on 1/18/2007 (item 11, page 5, last line, continuing on page 6, line 1) that Katsumata et al. clearly teach the type of aminotransferase encoded by the *C. glutamicum* gene used by Katsumata et al. It is reiterated herein that this information is clearly stated in the Abstract of that reference and on page 1, lines 21-22 of said reference. The *C. glutamicum* aminotransferase of Katsumata et al. has been **unequivocally** identified as an aspartate aminotransferase. Katsumata et al. not only refers to it by its **complete** name but it also provides its corresponding EC number, which is 2.6.1.1 (official name aspartate aminotransferase). Katsumata et al. refer to it using the

abbreviated form AAT throughout the entire document (page 1, lines 21-27). Thus, contrary to Appellant's assertions, Katsumata et al. teach (1) a protein having the same enzymatic activity as that of the polypeptide of SEQ ID NO: 2, and (2) the increase in L-threonine production as a result of increasing the synthesis of aspartate aminotransferase.

On pages 7-8 of the Brief, Appellant argues that neither Debabov et al., Edwards et al. or Kishino et al. make up for the deficiencies of Katsumata et al. because (1) Debabov et al. fail to teach the aspartate aminotransferase of SEQ ID NO: 2, (2) Edwards et al. is cited for disclosing that aspartate aminotransferase is effective for L-phenylalanine production in *E. coli* even though the L-threonine biosynthetic pathway is completely different from the L-phenylalanine biosynthetic pathway, and (3) Kishino et al. fail to teach the method of increasing expression of the gene of SEQ ID NO: 1 to increase L-threonine production. Appellant argues that neither the primary reference nor any of the secondary references teach L-threonine production in *E. coli* by increasing the expression of the gene of SEQ ID NO: 1. Appellant submits that the combination of the cited references does not suggest the claimed invention and points out that the connection between the increase in expression of the gene of SEQ ID NO: 1 and increased production of L-threonine is not remotely suggested by the instant references.

Appellant is reminded that the instant rejection is not an anticipation rejection but rather an obviousness rejection. As such, the Examiner has not relied on each of the references individually as teaching every aspect of the claimed invention. Instead, the Examiner has relied upon the combined teachings of the cited references to establish a *prima facie* case of obviousness.

The only differences between the teachings of Katsumata et al. and the claimed invention are: (1) the organism used, (2) the aspartate aminotransferase gene expressed, and (3) the use of low copy number vectors, or the use of the lac, trp or P_L promoters. The references cited by the Examiner provide those limitations which are missing in Katsumata et al. and also provide the required motivation to combine those references.

Debabov et al. was introduced by the Examiner as the reference which teaches the *E. coli* cell which meets all the limitations recited in the claims except for the expression of the gene of SEQ ID NO: 1, namely expression of the *E. coli* thrA gene, the *E. coli* thrB gene, the *E. coli* thrC gene and the *E. coli* rhtA gene. It is reiterated herein that the strain of Debabov et al. is the **same** strain Appellant transformed with plasmids containing the gene of SEQ ID NO: 1 for the production of L-threonine. Debabov et al. was not introduced as teaching the gene of SEQ ID NO: 1. Thus, the teachings of Debabov et al. provide the organism of the claimed invention.

Edwards et al. was introduced by the Examiner **primarily** as the reference which teaches a gene comprising SEQ ID NO: 1 and encoding the polypeptide of SEQ ID NO: 2. Thus, the teachings of Edwards et al. provide the *E. coli* aspartate aminotransferase gene of the claimed invention. While it is agreed that (1) Edwards et al. teach an *E. coli* cell transformed to express the *E. coli* aspC gene (SEQ ID NO: 1) for the production of aromatic amino acids and not L-threonine, and (2) the biosynthesis of aromatic amino acids and L-threonine are not directly associated, the teachings of Edwards et al. are still useful to show that the same gene has been linked to the production of other amino acids as well. However, as clearly stated in the original rejection and previous Office actions, the main motivation to express the gene of Edwards et al. in the *E. coli* strain of Debabov is found in the teachings of Katsumata et al.

Kishino et al. was introduced by the Examiner **primarily** to show that there is a high motivation to (1) use the strain of Debabov et al. for the production of L-threonine, and (2) use low copy number vectors and inducible promoters such as lac, trp, and P_L for expressing genes useful in the production of L-threonine. This reference was not introduced as teaching the claimed method. Thus, in addition to motivation, this reference provides the low copy number vectors and promoters of the invention.

Therefore, contrary to Appellant's assertions, the combined teachings of Katsumata et al., Edwards et al., Debabov et al. and Kishino et al. not only provide a connection between expressing the gene of SEQ ID NO: 1 and production of L-threonine but they also clearly suggest the claimed invention.

On page 8 of the Brief, Appellant argues that it would not be expected by the skilled artisan that an additional increase in L-threonine production would result by increasing the expression of the gene encoding the polypeptide of SEQ ID NO: 2 because threonine production is already optimized at a high level in a bacterium with increased expression of the thrA, thrB, thrC and rhtA genes. Appellant also argues that even if expression of the aspC gene is increased, the production of L-threonine would not also increase unless the supply of aspartic acid runs short in the whole pathway of threonine synthesis. It is stated that the shortage of aspartic acid will occur if the synthesis reaction of aspartic acid from oxaloacetic acid is the rate-limiting step. Since one of skill in the art would not have known which reaction is the rate limiting step in the synthesis of L-threonine, an increase in L-threonine production as a result of increasing the expression of aspC with thrA, thrB, thrC, and rhtA is completely unexpected.

The Examiner acknowledges that the art and the specification teach that the strain of Debabov, which expresses the thrA, thrB, thrC and rhtA genes, is a high L-threonine producer. However, as indicated in the Advisory Action mailed on 5/11/2007, there is no evidence that would lead one of skill in the art to conclude that no additional increase in the production of L-threonine could be accomplished by further modifying the strain of Debabov et al. It is reiterated herein that there is no evidence in the specification or the art suggesting that the L-threonine yields levels obtained with the strain of Debabov et al. are the maximum yields one could possibly obtain. Thus, there is the potential for improving L-threonine yields beyond those obtained with the strain of Debabov et al. Also, there is no indication in the specification or the prior art that increased expression of the thrA, thrB, thrC and rhtA genes would preclude additional synthesis of L-threonine if an additional modification is made to an *E. coli* cell that already is able to overexpress the thrA, thrB, thrC and rhtA genes. In the absence of a teaching or

suggestion indicating that additional modifications aimed at increasing the availability of precursors of L-threonine would either have no effect or impair the synthesis of L-threonine, one of skill in the art would have to conclude that there is a **reasonable** expectation that increasing the levels of a precursor of L-threonine would result in some additional synthesis of L-threonine.

The Examiner acknowledges that one of skill in the art could have considered the possibility of a "bottleneck" in the *E. coli* strain of Debabov et al. which would not allow an increase in L-threonine even if the synthesis of aspartic acid increases. However, the Examiner disagrees with Appellant's contention that one of skill in the art would have reasonably concluded that an increase L-threonine due to the increase in expression of the gene of SEQ ID NO: 1 is an unexpected and non-obvious result, particularly in view of (1) the results obtained by Katsumata et al. where it is clear that the increased expression of a gene encoding aspartate aminotransferase resulted in an increase in L-threonine, and (2) the lack of any teaching or suggestion indicating that such bottleneck exists or that increasing the expression of the aspC gene in the strain of Debabov et al. would be detrimental to the synthesis of L-threonine. While having additional information regarding the rate limiting steps in the production of L-threonine would have been useful, lacking that knowledge would have not deterred one of skill in the art to apply the teachings of Katsumata et al. for the production of L-threonine in *E. coli*. At a minimum, with the knowledge available to the skilled artisan at the time of the invention, one of skill in the art would have been motivated to increase the expression of the *E. coli* aspartate aminotransferase gene of Edwards et al. in the *E. coli* strain of Debabov et al.

The *E. coli* strain of Debabov et al. is an *E. coli* cell modified to increase the expression of several genes of the *E. coli* threonine operon (thrA, thrB, and thrC). These genes encode the enzymes aspartokinase I-homoserine dehydrogenase I, homoserine kinase, and threonine synthase, respectively, which are involved in the conversion of aspartate to threonine. The thrA gene used in the strain of Debabov et al. was a mutant gene encoding an enzyme which is not feedback inhibited by L-threonine.

This information was well known in the art before the invention was made as indicated in paragraphs [0005], [0006] and [0050] of the specification. Since the expression of these genes is increased in the strain of Debabov et al., one of skill in the art would have reasonably concluded that the additional synthesis of these enzymes would most likely result in those precursors which are substrates of these enzymes to be utilized at a higher rate than in an *E. coli* strain which lacks the modifications made by Debabov et al. In view of the fact that aspartate is one of the substrates of the enzyme encoded by the thrA gene, and the enzyme encoded by the thrA gene used by Debabov et al. is not feedback inhibited by L-threonine, it is expected that the aspartate pool would be depleted at a higher rate due to the increase in expression of the threonine operon genes. As such, the skilled artisan would have to assume that it is **more likely than not** that increasing the expression of the aspC gene would result in **some** increase in L-threonine production because the aspC gene encodes an enzyme which catalyzes the formation of aspartate, which is one of the precursors that is being depleted faster. Therefore, contrary to Appellant's assertions, prior knowledge of the bottlenecks in the synthesis of L-threonine would have not been required for one of skill in the art to reasonably expect some increase in L-threonine production due to an increase in the expression of the aspC gene in the strain of Debabov et al.

On pages 9-10 of the Brief, Appellant refers to a statement made by the Examiner in the Advisory action mailed on 5/11/2007 where it is indicated that the art, as evidenced by the specification on page 3, paragraph [10], teaches that a similar approach (increased expression of the aspC gene) was followed in the production of L-lysine in *E. coli*, which is also synthesized from aspartic acid, that resulted in increased production of L-lysine. Appellant asserts that this statement is not correct. According to Appellant, Kojima et al. contains Tables 8, 10, 12, 14, 18, 19, 20 and 21 which show the effect of increasing the expression of various genes on the production of L-lysine including aspC. Appellant points out that these tables show that production of L-lysine is hardly increased in all strains transformed with a plasmid containing the aspC gene (paspC) and that there is no increase in L-lysine production in

strains where the supply of aspartic acid is not rate-limiting for L-lysine. Appellant submits that since the rate-limiting step in the production of L-threonine is unknown, it would not have been obvious to one of ordinary skill in the art that increasing the expression of the aspC gene would lead to increased threonine production. Appellant argues that the invention disclosed is the first to show an increase in L-threonine production by combining the expression of aspC, thrA, thrB, thrC, and rhtA genes.

For the record, the art cited by Appellant in the specification (paragraph [0010]) is U.S. Patent No. 6,040,160 (Kojima et al.), which is the reference provided by Appellant as Exhibit B. This reference was not was not a new reference introduced to the record by the Examiner in the Advisory action but a reference which Appellant refers to in the specification (paragraph [0010]). The Examiner refers to its teachings to show (1) Appellant's own admission of the state of the art at the time of filing, and (2) to further support the argument that the prior art provides more than enough motivation for one of skill in the art to combine the references originally cited in the rejection.

With regard to arguments that the statement made by the Examiner is "simply not correct" as asserted by Appellant, it is noted that paragraph [0010] of the specification states:

> "The effect of amplification of *aspC* gene on production of L-lysine - an amino acid of aspartate family - is disclosed. Amplification of *aspC* gene was used for L-lysine production by *E. coli* (US patent 6,040,160). Coryneform bacteria harboring an aspartokinase and enhanced DNA sequence coding for several enzymes including aspartate aminotransferase was used for L-lysine production (US patent 6,004,773)."

Thus, it is evident from Appellant's own specification that the strategy of increasing the expression of the aspC gene for the production of another amino acid of the aspartate family (i.e., L-lysine) has been disclosed previously by Kojima et al. As known in the art, L-threonine is also a member of the aspartate family of amino acids. It is Appellant and not the Examiner who has stated what the teachings of the prior art were regarding increasing the expression of the aspC gene and the production of L-lysine. Furthermore, Table 8 of Kojima et al. shows that when the expression of the aspC gene was increased there was a 50% increase in the production of L-lysine (0.08 g/L vs. 0.12 g/L). Similarly, Table 18 of Kojima et al. shows a 50% increase in the production of L-lysine (0.08 g/L vs. 0.12 g/L) when the

expression of the aspC gene is increased. Kojima et al. clearly teach examples where increases in the expression of the aspC would result in increases in the synthesis of L-lysine. Therefore, in view of the record, the Examiner does not believe that the statements made were "simply not correct" as Appellant contends.

The Examiner acknowledges that Kojima et al. discloses examples where increasing the expression of the aspC result in little or no increase in L-lysine production. As suggested by Appellant, it appears that this little or no effect on L-lysine production is due to the fact that for certain gene combinations, the production of aspartate was not the rate-limiting step. As previously acknowledged by the Examiner, one of skill in the art would have considered the possibility of a bottleneck in the production of L-threonine wherein an increase in aspartate would not have resulted in an increase in L-threonine. However, neither the lack of knowledge as to which was the rate limiting step in the production of L-threonine, nor the results showing little or no increase in L-lysine production would have deterred one of skill in the art to combine the teachings of Katsumata et al., Debabov et al., Edwards et al., and Kishino et al. because (1) Kojima et al. also disclose examples of increases in L-lysine production when the expression of the aspC gene is increased, and (2) Katsumata et al. teach the same method as claimed with a different microorganism.

While Appellant's arguments could have been persuasive if the claims recited a specific level of L-threonine increase in production, Appellant is reminded that the **claims do not require an increase in L-threonine production** compared to the production of L-threonine when there is no increased expression of the aspC gene. All that is required by the claims is the **production of L-threonine** by the recited *E. coli* cell. Appellant has stated in the specification that the strain of Debabov et al. is a high L-threonine producer. Thus, even if the increased expression of the aspC would have not resulted in an increase in L-threonine, one of skill in the art would still have reasonably expected that the strain of

Debabov et al. further modified to increase the expression of the aspC could be cultured to produce L-threonine, which is **all** that is required by the claims.

The Examiner recognizes that there is no absolute certainty that an increase in L-threonine production could be achieved by increasing the expression of the *E. coli* aspC gene taught by Edwards et al. in the *E. coli* strain of Debabov et al. However, it is abundantly clear from the art that there is not only motivation but a **reasonable** expectation of success at increasing production of L-threonine by increasing the expression of the aspC gene. Therefore, for the reasons of record and those set forth above, the invention as a whole would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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